Studies on the Lipid of "Ruvettus pretiosus" I. The Composition of AIcohols and Fatty Acids

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I. The Composition of Alcohols and Fatty Acids

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Summary

1. The fatty acid compositions of the various tissue oils of *Ruvettus pretiosus* were determined by gas liquid chromatography and were found to be mostly composed of oleic, plamitoleic and eicosaenoic acids in the flesh, skin and bone and oleic, docosahexaenoic and stearic acids in the heart and liver.
2. The ratio of saponifiable matter to the unsaponifiable matter ranged 40 to 45 per cent in the flesh, 24 per cent in the bone and 3.7 per cent in the liver.
3. Hydroxy acid was absent from the lipids of all tissues of *Ruvettus pretiosus*.
4. The alcohol compositions of Ruvettus pretiosus were determined by gas liquid chromatography and were found to be mostly composed of cetyl, oleyl and palmitoleyl alcohols.

Several investigations have been made on the lipid *Ruvettus pretiosus* Cocco, a fish of the *Acinaceidae* family (=*Gemplidae*). In 1925 Gudger (1) found that the oil contained a purgative principle in the flesh. Kimura (1926) (3) reported that it was largely composed of cetyl alcohol accompanying with a small amount of oleyl alcohol. According to Cox and Reid (1932) (2), the wax was found to be chiefly composed of cetyl and oleyl oleate. They also suggested that the purgative properties of the lipid may be due to the presence of hydroxyoleic acid which was supposed to be an isomer of ricinoleic acid and composed approximately 13 per cent of the total fatty acids. Recently, Nevenzel et al. and Mori et al. (5, 6) attempted independently the quantitative analysis of fish lipid using gas liquid chromatography and both of them obtained negative results as to the presence of hydroxyoleic acid. Thus they considered that the purgative proporties were dependent on the high contents of wax in the meat. Latter authors made a feeding experiment with the fish meat on cats and rats and found that higher alcohols were primarily resposible for an active purgative and caused a high mortality of the animals when raised to 1.5 per cent of the diet.

Although the catch of this fish is small in Japan, the Japanese are sometimes eat it as "Sashimi" (raw meat) or "Misozuke" (pickles with fermented soybean

paste). Moreover, there are some peoples in western Japan that often eat the roasted skin of sperm whale containing more than 20 per cent oil, of which higher alcohols are predominant, as a side-dish, Oden. If the higher alcohols are purgative as already pointed out, it is more probable that the person who eats too much of the meat has diarrhea or feels sick at the stomach. For the attempt to make the nutritive value of marine oils clear, especially in relation to their higher alcohols and seborrhea of animals we feel a need of further investigations on their purgative and toxic properties. We have carried on the feeding experiments with rats using oils from various fishes and marine animals. First the present paper deals with the results conducted by the authors on the lipid analysis of various parts of *Ruvettus pretiosus* using gas liquid chromatography.

Experimental Method

1. Materials and Preparation of Sample.

Material used was a fish, *Ruvettus pretiosus*, which was caught by a long-line off the coast of Okinawa, in May of 1965, landed at Yaizu, Shizuoka-Prefecture, and kept frozen in the cold storage there before use. It was 6 Kg in weight and 83 cm in length. The various portions were cut off from the dorsal-, ventral-, tail- and dark-meat and the outer part and inner part of the dorsal meat and skin, bone, heart and liver respectively. They were ground in a blender, after cutting small pieces, treated three times with acetone, and followed by extraction with diethyl ether. The combined extracts were washed with water and dried over anhydrous Na_2SO_4. After removing off the solvent in vacuo, the residual lipids were saponified with ethanolic-potassium hydroxide by the usual method. The fatty acids were converted into methyl esters by diazo-methane. The alcohols of the unsaponifiable part were also converted to the acetates by pyridine-anhydrous acetic acid. Both of them were used as samples for the gas liquid chromatography. The hydrogenation of fatty acids and alcohols was carried out using 5 per cent Pd on charcoal as a catalyst in a ethanol-ether solution.

2. Gas Liquid Chromatography Technique.

The instrument used was a Shimazu HFD-I with a flame ionization detector. The column of 3 m in length was packed with 80～100 mesh Diasolid L (Nippon Chromato Co. Ltd.) coated with 15 per cent ethylene glycol succinate polyester. The operating condition was as follows: column temperature 197°C: carrier gas nitrogen.

The quantitative values of each component were determined from the areas of each peak evaluated by the triangulation procedure. The identification of the component was done by applying the graphical method between the number of carbon atom of alcohol or fatty acid and retention time. With respect to the

saturated or unsaturated constituents, we identified them by observing the peak concerned on chromatogram before and after hydrogenation.

Result

1. General Component.

As shown in Table 1, the flesh contained from about 13 to 18 per cent of the lipid. It is interesting to note that the lipid content of *Ruvettus pretiosus* is higher in dorsal meat than in ventral one. Comparing the outer part of meat to the inner one, the former was generally higher than in the latter. The skin and bone contained a large amount of lipid ranging about from 20 to 22 per cent. It is worthy of notice that Cox and Reid observed a high purgative activity of bone oil. The liver oil was found to be 19.1 per cent which were lower than the value of 26.1 per cent obtained by Nevenzel et al. This is due to the different seasons and feeding conditions. The oil content is very low in the heart. The ratio of saponifiable to unsaponifiable matter was 55 to 60 per cent in the flesh and 61.5 to 75.6 per cent in the skin and bone. As to the liver it was remarkably high, 96.3 per cent. As mentioned below this means that the liver lipid mostly consists of triglyceride.

TABLE 1. *General component of the tissue of "Ruvettus pretiosus".*

part	Moisture (%)	Protein (%)	Ash (%)	Fat (%)	Sap.matter⊛	Unsap. matter⊛
Dorsal meat	59.94	23.57	1.08	16.45	58.7	41.3
Ventral meat	60.69	18.38	0.96	13.63	55.1	44.9
Tail meat	66.43	19.38	0.99	12.61	58.4	41.6
Outer part of meat (from surface to 5mm)	60.00	17.69		17.93	55.6	44.4
Inner part of meat (inside from 45 to 50mm)	63.84	19.31	0.95	14.72	60.1	39.9
Dark meat	63.48	17.44	0.93	15.65	55.0	45.0
Skin	50.82	18.63	5.04	21.76	61.5	38.5
Bone	55.62	11.75	8.31	20.60	75.6	24.4
Liver				19.10	96.3	3.7
Heart	84.83			2.10	63.0	37.0

⊛ % in 100g of oil.

2. Composition of Fatty Acids.

The fatty acid compositions between the lipids of various parts of the fish except the liver and heart showed a great similarity. The percentage of oleic acid was more than 60 per cent except for the liver and heart. The contents of eicosaenoic, linoleic and palmitoleic acids were comparatively high. There occurred

TABLE 2. *The fatty acid composition of "Ruvettus pretiosus" lipid.*

Fatty acid	Dorsal meat	Ventral meat	Tail meat	Outer part of meat	Inner part of meat	Dark meat	Skin	Bone	Liver	Heart
C 12:0	trace	trace	trace	trace	trace	trace	trcce	trace	trace	trace
C 14:0	0.1	0.3	0.2	0.2	0.2	0.3	0.2	0.2	0.7	0.6
C 15:0	0.2	trace	0.1	trace	trace	0.1	trace	trace	0.2	0.2
C 15:1	trace	0.3	trace	trace	0.5	trace	0.8	1.1	0.1	0.1
C 16:0	1.3	1.4	1.4	1.0	1.5	2.1	1.2	1.1	11.7	11.7
C 16:1	3.5	4.3	4.2	3.6	3.4	3.3	4.2	2.7	5.4	3.4
C 17:0	1.5	1.7	1.7	1.5	1.5	1.8	1.4	1.0	2.2	1.7
C 17:1	1.2	1.5	1.4	1.2	0.9	1.0	2.4	2.6	1.4	0.9
C 18:0	0.6	0.5	0.6	0.6	trace	0.7	0.3	0.0	3.7	5.5
C 18:1	66.7	60.2	68.7	68.1	65.3	68.9	67.1	72.7	39.9	31.6
C 18:2	5.8	6.3	6.0	6.4	4.8	4.5	4.7	5.2	3.2	2.4
C 20:1	8.8	11.1	10.5	10.3	9.4	9.8	7.6	8.9	6.6	4.8
C 20:2	0.2	0.3	trace	0.1	trace	trace	0.3	0.1	0.3	0.6
C 20:3	0.3	0.2	0.1	0.1	trace	0.2	trace	trace	0.1	1.1
C 20:4	2.3	2.8	2.0	2.5	2.4	2.1	2.4	1.6	2.4	4.6
C 20:5	0.5	0.6	0.2	0.4	0.6	0.2	0.7	0.4	1.8	0.6
C 22:0	0.9	1.1	0.6	0.8	1.0	0.6	1.5	0.7	1.7	2.7
C 22:1	0.5	0.9	0.3	0.5	1.0	0.6	0.6	0.2	0.6	7.3
C 22:5	1.5	1.3	0.6	0.7	2.2	1.0	0.9	0.7	1.0	1.5
C 22:6	3.6	4.9	1.0	1.8	5.1	2.8	3.2	2.1	11.4	19.2
C 24:0	0.5	0.5	0.2	0.2	0.3	0.1	0.3	0.0	5.2	1.0

TABLE 3. *The alcohol composition of "Ruvettus pretiosus" lipid.*

Alcohol	Dorsal meat	Ventral meat	Tail meat	Outer part of meat	Inner part of meat	dark meat	Skin	Bone	Liver	Heart
C	0.1	0.1	0.1	trace	trace	0.1	trace	trace	trace	trace
C	0.1	0.1	0.1	trace	trace	trace	trace	trace	1.2	trace
C 14:0	3.5	3.5	3.1	3.6	3.6	3.3	3.8	3.2	2.6	3.1
C 14:1	0.9	0.6	0.3	0.8	1.0	1.0	1.1	0.9	0.9	1.0
C 15:0	1.4	1.5	1.2	1.3	1.4	1.3	1.3	1.2	1.2	1.5
C 15:1	0.6	0.5	0.5	0.6	0.6	0.8	0.7	0.6	0.6	0.6
C 16:0	49.4	39.9	39.7	50.0	39.6	37.9	43.8	49.0	27.7	44.0
C 16:1	8.6	10.8	12.0	9.1	12.8	13.1	9.5	7.6	9.4	8.6
C 16:2	4.4	5.0	4.5	4.0	4.8	4.8	5.6	4.7	4.0	4.9
C 17:1	2.9	2.7	2.6	2.4	3.0	2.6	3.2	2.9	2.4	2.0
C 18:0	7.4	8.2	8.4	6.1	7.5	7.1	7.2	6.2	5.1	8.0
C 18:1	15.9	19.2	19.6	17.7	18.7	22.4	17.6	16.6	20.5	18.2
C 19:1	2.4	2.2	1.9	1.4	3.4	2.9	2.9	3.6	2.2	2.6
C 20:0	0.3	0.8	0.9	0.8	0.4	0.7	0.6	0.6	trace	0.6
C 20:1	2.8	4.9	4.9	2.6	3.4	4.2	2.7	2.8	8.1	3.0
C 22:0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	trace	trace
C 22:2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	14.3	0.9
C 24:0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	trace	0.7

30 to 40 per cent of oleic acid, 11 to 19 per cent of docosahexaenoic acid and 4 to 5 per cent of stearic acids and a small amount of palmitoleic and eicosaenoic acid in the liver and heart. It was found that the odd numbered fatty acids such as C_{15}- and C_{17}- acids were present in all tissues with some exceptions of the former acid.

3. On the Hydroxy Fatty Acids.

The occurrence of hydroxy acid in the *Ruvettus pretiosus* lipid is quite necessary for the affirmation of the result obtained by Cox et al. in 1932. Therefore we attempted to separate the hydroxy acid fraction from other fatty acids of the lipid employing the thin layer chromatography. As shown in Fig. 1, we could not find the spot at Rf 0.72 of ricinoleic acid. Hence our views concide with those of Nevenzel et al. and Mori et al. that denied the presence of the hydroxy acid in the fish lipids tested.

4. Composition of Alcohols.

The compositions of fatty alcohols in the lipid are summarized in Table 3. The content of cetyl alcohol attained to from 40 to 50 per cent of the total alcohols. Oleyl and palmitoleyl alcohols were 15.9 to 22.4 and 7.6 to 13.1 per cent, respectively. The dorsal meat and outer layers of meat were composed of about 10 per cent of

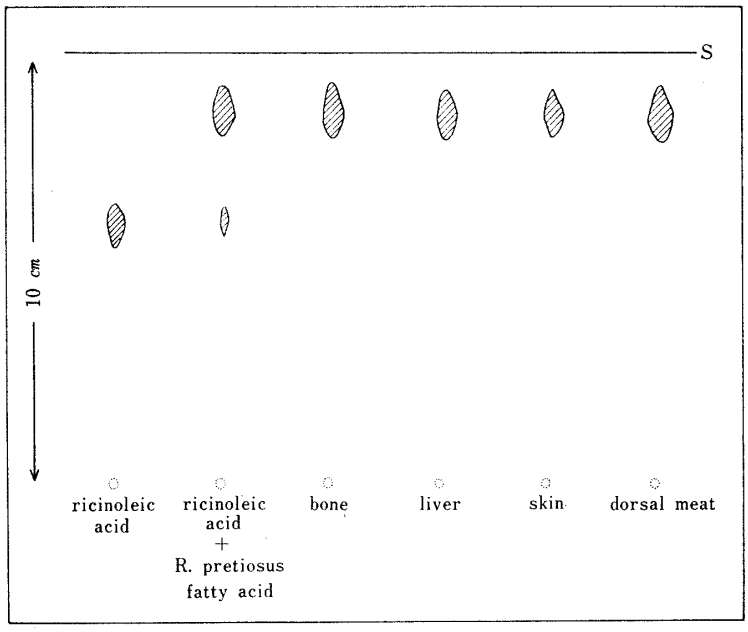

FIG 1. Thin layer chromatography of fatty acid and hydroxy acid.
Plate: silica gel-florisil (50: 50) activated at 140°C for 2 hours.
Solvent system: isopropyl ether-acetic acid (96: 4).
S: solvent front.
Developing agent: 50% sulphuric acid.

cetyl alcohol which is more than in other parts. On the contrary the contents of palmitoleyl, stearyl and eicosenyl alcohols were smaller than in other parts. The liver and heart lipids consisted of $C_{22:0}$, $C_{22:2}$ and $C_{24:0}$ alcohols which were not found in other parts. The alcohols of the odd carbon number such as C_{15}, C_{17} and C_{19} were present in the lipids of all tissues examined.

Discussion

The fatty acid composition of *Ruvettus pretiosus* lipid analysed by gas liquid chromatography was different from that of ordinary fish lipid. However, the substance supposed to be hydroxyoleic acid by Cox et al. was not found in this fish. Subsequently the authors conclude that the purgative properties of *Ruvettus pretiosus* do not depend on the hydroxy acid itself. On the contrary the alcohol compositions were very similar to those of the lipids of the sperm whale and beaked whale containing 17 to 28 per cent of cetyl alcohol and 48 to 57 per cent of palmitoleyl alcohol. As already reported by Cox et al. and Nevenzel et al., it was said that the purgative properties of *Ruvettus pretiosus* were due to the high content of these higher alcohols. Although the roasted blubber of sperm whale which contains much of these alcohols are used as a side-dish, "Irikawa" in Japan, one has never heard of a person who ate Irikawa having diarrhea. So, there are questions as to whether or not the purgative properties depend on a higher alcohol content alone.

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